

183. Are the Known Δ^2 -Cephems Inactive as Antibiotics because of an Unfavourable Steric Orientation of their 4α -Carboxylic Group? Synthesis and Biology of Two Δ^2 -Cephem- 4β -carboxylic Acids

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Two representatives of the yet unknown type of Δ^2 -cephem- 4β -carboxylic acids were prepared. Contrarily to the prediction based on the activity model of Cohen, both acids proved inactive as antibiotics. Possible reasons for this discrepancy are briefly discussed.

Introduction. – It has been suggested by Tipper and Strominger (1965) [1] that the antibiotic activity of penicillins is based on their ability to acylate, by virtue of their reactive β -lactam grouping, the transamidases involved in the biosynthesis of the bacterial cell wall [2] [3].

This chemical interpretation of the mode of action was subsequently extended to other known β -lactam antibiotics and was accepted by chemists active in this area as working hypothesis for the conception of new potential antibiotics. Along these lines, a great variety of compounds with chemically activated β -lactam moieties was synthesized leading to the discovery of some β -lactam systems of high interest; the penems are a typical example of the fruits of this effort.

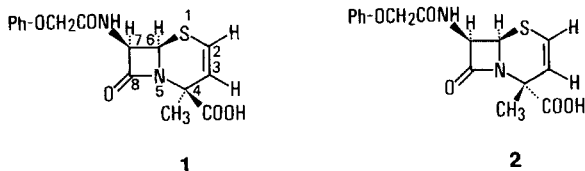
However, failures of this hypothesis were also reported revealing serious deficiencies of the reactivity model. Systematic analyses showed that the chemical reactivity of the β -lactam cannot be simply correlated with the biological properties of the molecules. Frère *et al.* [4] studied a wide range of β -lactam compounds and measured not only their chemical reactivity in alkaline hydrolysis but also the kinetic parameters of their interaction with various β -lactamases and peptidases. As a conclusion of these studies, it has been suggested that the primary parameter which governs the biological activity is the goodness of fit with the enzyme and not the chemical reactivity of the β -lactam.

In 1983, Cohen [5] analyzed the three-dimensional (3-D) features of a set of representative, biologically active and inactive β -lactam structures and concluded that highly specific 3-D recognition sites may be involved in the enzymes in their recognition of the antibiotics. Thus, a lack of antibacterial activity can be the result of a non-recognition rather than of an insufficient reactivity of the β -lactam system. According to this model, *e.g.*, the biological inactivity of Δ^2 -cephems may not be primarily the result of a low reactivity of their β -lactam grouping – in fact, as shown by Frère *et al.* [4], the liabilities for hydrolysis of Δ^2 - and Δ^3 -cephems are comparable – but rather a consequence of a misfit with the enzyme due to the unfavourable sterical location of the α -oriented

carboxylic group. In 4β -epimers, on the other hand, the acid function would fit very well with the 3-D requirements of the model of *Cohen*, and he postulated antibiotic activities for such compounds.

However, no Δ^2 -cephem acids with a β -orientation of the carboxylic group are known in the literature. Therefore, to check the correctness of the above mentioned challenging postulate, we have now prepared two Δ^2 -cephem- 4β -carboxylic acids and, for comparison of the biological activities, also their α -oriented counterparts.

Synthesis of 4α -Methyl- 7β -(2-phenoxyacetamido)- Δ^2 -cephem- 4β -carboxylic Acid and of its C(4) Epimer. – The fact that all known Δ^2 -cephem-4-carboxylic acids belong to the 4α -series suggests a greater thermodynamic stability of these compounds as compared to the – up to now hypothetical – 4β -epimers¹⁾. Therefore, to exclude any isomerizations at the C(4) center, we decided to fix the configuration at this position by substituting a Me group for the H-atom, *i.e.*, to synthesize the two epimeric 4-methyl- Δ^2 -cephem acids **1** and **2**.



For the synthesis of both compounds, the 3-hydroxy- Δ^3 -cephem ester **3**, an intermediate in the synthesis of the antibiotic *Oraspor*, was chosen as starting material [7]. By treatment of **3** with an excess of MeI in acetone in the presence of K_2CO_3 , two C(4)-methylated products **4** and **5** were mainly formed and isolated in 57 and 16.5% yield, respectively, after chromatographic separation. The structure assignment followed unequivocally from 1H -NMR analyses and NOE measurements and was later confirmed by the transformation products of both compounds.

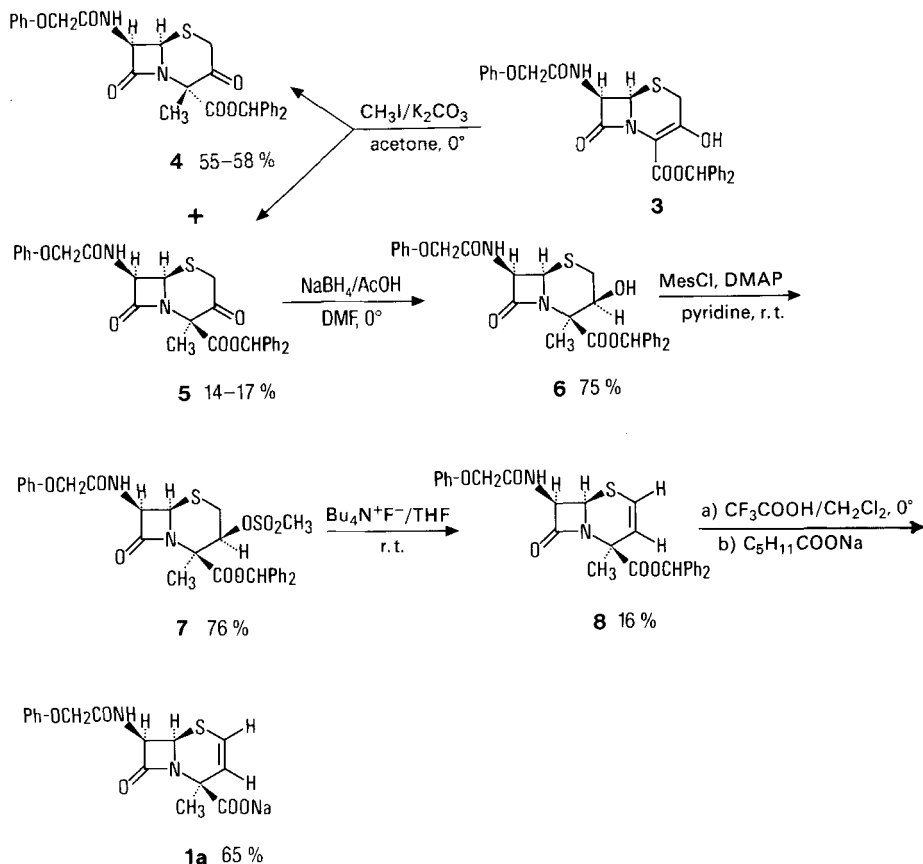
The minor, crystalline methylation product **5** with its β -oriented ester grouping was transformed into the sodium salt of 4α -methyl- 7β -(2-phenoxyacetamido)- Δ^2 -cephem- 4β -carboxylic acid (**1a**) in the following way (*cf. Scheme 1*). A $NaBH_4$ reduction of **5** in DMF in the presence of AcOH afforded the 3β -hydroxy derivative **6** (70–75% isolated yield); it was accompanied by a small amount of the 3α -isomer **9** and by variable amounts of the β -lactam-free lactone **10**. The latter compound is a secondary product formed from the alcohol **6**, the axial OH group of which being extremely well located for an intramolecular interaction with the β -lactam-carbonyl moiety.

The above mentioned trend of the alcohol **6** for intramolecular ring-opening of the β -lactam moiety created problems in its transformation to the mesylate **7**. Finally, the formation of the undesired lactone **10** was substantially suppressed by using a large excess (6–10 equiv.) of mesyl chloride in pyridine in the presence of 4-(dimethylamino)pyridine (DMAP) as catalyst (yield of **7**: 76%).

Unexpected problems of a different kind were met in the conversion of the mesylate **7** to the Δ^2 -cephem ester **8**, this in spite of the axial position of the mesyloxy grouping

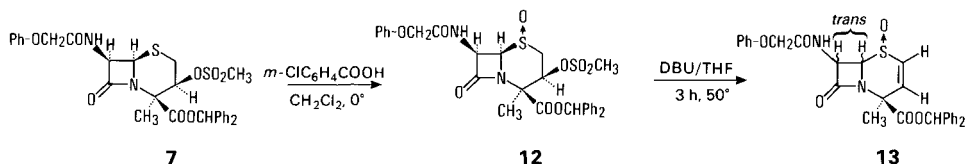
¹⁾ According to molecular-mechanics calculations [6], the 4α -carboxylic acids are more stable than their 4β -epimers by *ca.* 1 kcal/mol of energy difference.

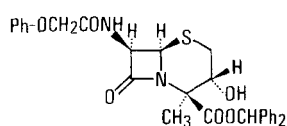
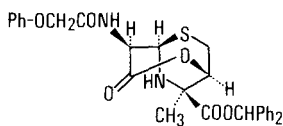
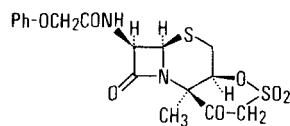
Scheme 1



favourable for such a *trans*-elimination reaction. Of the various examined elimination methods, only the use of anhydrous tetrabutylammonium fluoride in THF was partially successful allowing the isolation of **8** in yields of 15–20%; especially with extended reaction times, two other products prevailed: an isomer of **8** with *trans*-oriented β -lactam H-atoms, and the tricyclic keto sulfonate **11**, the latter a result of a primary deprotonation of **7** on the mesylate Me group²⁾.

²⁾ An attempted preparation of the corresponding *tosylate* from the alcohol **6** failed owing to the above mentioned, easy β -lactam ring-opening of **6** to the lactone **10**. An additional acidification at C(2) for deprotonation by transforming the mesylate **7** into the *S*-oxide **12** resulted, with DBU in THF in the elimination step in a complete *cis* $\rightarrow$ *trans* isomerization on the β -lactam moiety (cf. **13**).

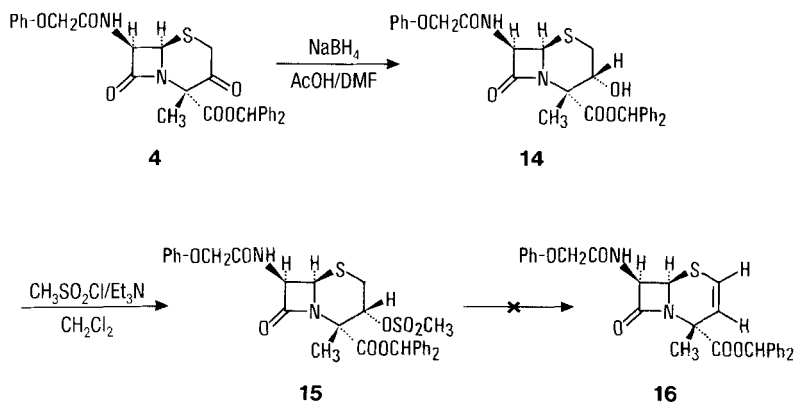


**9****10****11**

Finally, treatment of the benzhydryl ester **8** with CF_3COOH (in CH_2Cl_2) and an exchange of the free acid thus formed with sodium hexanoate afforded the sodium salt **1a** of the desired 4β -carboxylic acid. Its structure was confirmed, in addition to other spectroscopic evidence, by a strong NOE intensity enhancement for the H-atom at C(6) upon irradiation of CH_3 at C(4)³.

For the preparation of the – also unknown – 4β -methyl- Δ^2 -cephem acid **2** with the α -oriented carboxylic group, a similar scheme as described for **1a** was considered, the starting material this time being the major methylation product of **3**, namely the 4β -methyl-3-oxocephem ester **4** (Scheme 2).

Scheme 2



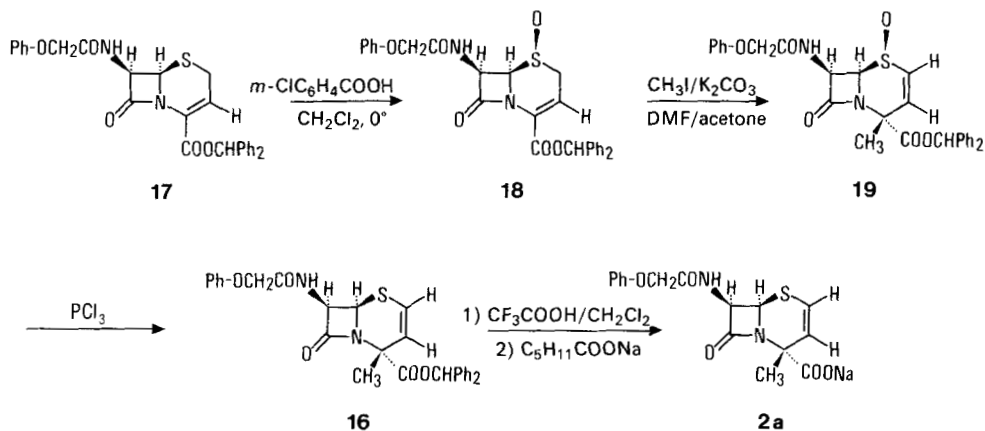
However, in contrast to the reduction of ketone **5** to the axial alcohol **6**, a similar NaBH_4 reduction of the epimeric ketone **4** (in AcOH/DMF) gave the equatorial, 3α -oriented alcohol **14** and the following mesylation the equatorial mesylate **15**⁴). All attempts to eliminate the elements of methansulfonic acid from the latter compound led only to intractable decomposition products and no Δ^2 -cephem ester **16** could be identified.

On the other hand, as shown in Scheme 3, the ester **16** was easily prepared starting from the benzhydryl ester **17** of 7β -(2-phenoxyacetamido)- Δ^3 -cephem-4-carboxylic acid [7a] [8]. The Δ^3 -cephem *S*-oxide **18**, prepared from **17** by treatment with 1 equiv. of *m*-chloro-

³) For the isomeric salt **2a** (see below), no NOE was observed.

⁴) A conformational analysis of the 4-methyl ketones **4** and **5** reveals, that the most stable conformation of **4** has the six-membered ring in a chair and that of **5** in a distorted boat form, the Me and the ester groups on C(4) being – in both cases – equatorial and axial, respectively. The nucleophilic attack of the hydride reagent on the C(3) carbonyl takes place from the face opposite to that where the ester grouping is located and leads, with **4**, to the formation of the equatorial 3α -alcohol **14** and, in the case of **5**, to the axial 3β -alcohol **6**.

Scheme 3



perbenzoic acid (CH_2Cl_2 , 0°), was methylated with an excess of MeI in acetone/DMF in the presence of K_2CO_3 to give exclusively the 4 β -methyl- Δ^2 -cephem derivative **19**. Deoxygenation of the *S*-oxide grouping in **19** by PCl_3 then afforded the ester **16**. This nicely crystalline compound suited well for an X-ray analysis which confirmed, among other features, the β -orientation of the Me group on C(4) (*cf. Figure and Exper. Part*). For

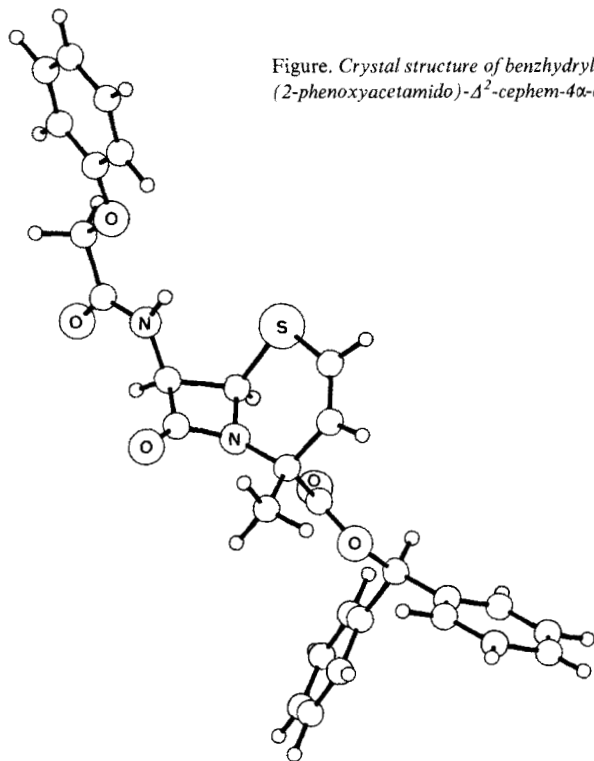
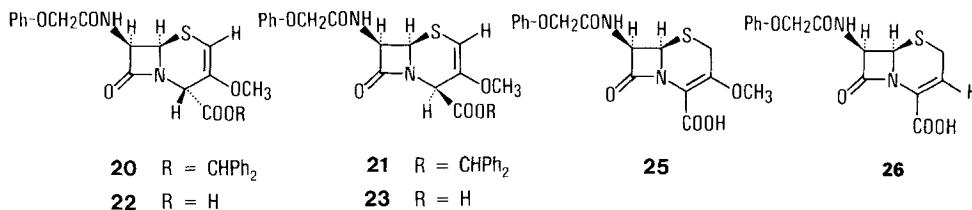


Figure. Crystal structure of benzhydryl 4-methyl-7 β -(2-phenoxycarbonylamido)- Δ^2 -cephem-4 α -carboxylate (**16**)

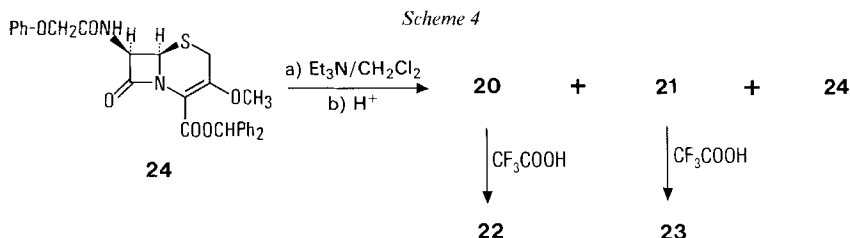
biological tests, the ester **16** was converted – with CF_3COOH and sodium hexanoate – to the sodium salt **2a** of the corresponding acid.

Synthesis of 3-Methoxy-7 β -(2-phenoxyacetamido)- Δ^2 -cephem-4 β -carboxylic Acid and its 4 α -Epimer. – In the synthesis of 3-methoxy-substituted cepheids, a minor by-product was detected in one of the steps which was isomeric with the Δ^2 -cephem-4 α -carboxylate **20** and was identified – mainly by $^1\text{H-NMR}$ analysis⁵) – as the 4 β -epimer **21** [9].



In this observation, we recognized a chance to prepare a pair of Δ^2 -cephem-4-carboxylic acids without any further substitution on C(4), *i.e.* the acids **22** and **23** which would be true isomers of the biologically active Δ^3 -cephem-4-carboxylic acid **25**. The geometrical features of the acid **23** should fully comply with the demands of the model of *Cohen*.

Equilibration of the 3-methoxy- Δ^3 -cephem ester **24** [7a] in CH_2Cl_2 with Et_3N afforded, after acidic quenching, a mixture of **24** (*ca.* 23%) and of the two Δ^2 -cephem esters **20** (*ca.* 75%) and **21** (*ca.* 2%; *cf.* Scheme 4). Both Δ^2 -cephem esters were isolated in pure form by column chromatography and by crystallization. Separate removal of the benzhydryl group ($\text{CF}_3\text{COOH}/\text{CH}_2\text{Cl}_2$) then gave the corresponding acids **22** and **23**. Unfortunately, the 4 β -carboxylic acid **23** – the most important compound for the planned biological testing – crystallized from all solvents tried in very fine needles unsuitable for an X-ray structure determination; however, its structure as well as that of the isomeric acid **22** and of the corresponding esters **21** and **20** follow unequivocally from their $^1\text{H-NMR}$ analyses.



Biological Results and Discussion. – Both pairs of Δ^2 -cephem-4-carboxylic acids **1/2** and **22/23** were tested for biological activity in an agar dilution test; the Δ^3 -cephem-4-carboxylic acids **25** and **26** were also included in the tests as standards.

As shown in Table 1, the hopes for a prominent biological activity of the Δ^2 -cephem-4 β -carboxylic acids (see **1a** and **23**) as postulated by *Cohen* were not fulfilled. The

⁵) A long-distance coupling (over 5 bonds) with $J \approx 1$ Hz is observed in **21** between $\text{H-C}(4\alpha)$ and $\text{H-C}(7\alpha)$; such a coupling is absent in the $^1\text{H-NMR}$ spectra of **20** and of the known Δ^2 -cephem-4 α -carboxylic acids with β -oriented $\text{H-C}(4)$.

Table 1. *Biological Activity of Δ^2 -Cephem-4-carboxylic Acids 1, 2, 22, and 23 in Agar Dilution Test^{a)}*

	1a	2a	26	23	22	25
<i>Staphylococcus aureus</i> 10 B	> 128	32	0.2	> 128	16	1
<i>Staphylococcus epidermidis</i> R 13	> 128	16	0.2	> 128	8	1
<i>Streptococcus pyogenes</i> Aronson	> 128	64	1	128	32	< 1
<i>Streptococcus pneumoniae</i> III/84	> 128	32	2	128	32	2
<i>Neisseria meningitidis</i> 1316	> 128	128	2	> 128	128	4
<i>Neisseria gonorrhoeae</i> 1317/4	> 128	64	2	> 128	32	4
<i>Haemophilus influenzae</i> NCTC 4560	> 128	> 128	4	> 128	128	4
<i>Clostridium perfringens</i> 194 anaerob	> 128	32	1	32	4	2
<i>Bacteroides fragilis</i> L 01 anaerob	> 128	> 128	32	> 128	> 128	128
<i>Escherichia coli</i> 205	> 128	> 128	32	> 128	> 128	> 128
<i>Klebsiella pneumoniae</i> 327	> 128	> 128	32	> 128	> 128	> 128

^{a)} DST agar as test medium; inoculum 10^4 /drop, incubation temp. 37°; MIC in $\mu\text{g/ml}$.

4-methyl-substituted compound **1a** proved entirely inactive against all microorganisms used in the test, and the 4-unsubstituted 4β -acid **23** displayed only a marginal activity against three strains. In both cases, the activity of the 4β -oriented acid was inferior to that of the Δ^3 -cephem acids **26** and **25**, and even to that of the very weakly active Δ^2 -cephem- 4α -carboxylic acids (see **2a** and **22**). The relatively high stability of the Δ^2 -acids as determined at 37° in a biological buffer at pH 7.4 excludes an explanation of the observed, more or less negative results by decomposition of the substances in the early stages of the biological test.

Thus, it has to be concluded that an activity model based solely on a good fit of the β -lactam compound with the active site of the transpeptidase fails to explain the above mentioned biological results. On the other hand, we feel it incorrect to reject, because of the biological inactivity of the two β -oriented Δ^2 -cephem acids, the conclusions about geometrical requirements for antibacterial activity of β -lactam antibiotics as presented in the quoted paper by Cohen [5]. Considering this failure of the steric model on the one hand, and the difficulties encountered with the reactivity model on the other hand, we now tend to believe – this somewhat contrarily to the conclusions of Frère *et al.* [4] – that for a good antibiotic activity a subtle combination of both features, namely a 3-D recognition and a sufficient chemical reactivity of the β -lactam, are necessary. The latter condition is obviously not fulfilled in the otherwise geometrically well suited Δ^2 -cephem- 4β -carboxylic acids.

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Experimental Part

General. R_f values: Merck silica gel 60 F_{254} TLC plates. M.p.: Kofler; uncorrected. IR spectra: absorptions in cm^{-1} , $^1\text{H-NMR}$ (400.1 MHz) and $^{13}\text{C-NMR}$ (100.6 MHz): Bruker WM 400 spectrometer; some spectra were recorded on Bruker AM 360 and on Varian HA 100B spectrometers; chemical shifts as δ values in ppm with respect to tetramethylsilane as internal reference ($\delta = 0$ ppm), coupling constants J in Hz. MS: Varian CH 7 spectrometer; FAB measurements: ZAB-HF spectrometer of VG Analytical.

Benzhydryl 4-Methyl-3-oxo-7 β -(2-phenoxyacetamido)cepham-4-carboxylates⁶⁾ (4 and 5). In a soln. of 20.66 g (40 mmol) of benzhydryl 3-hydroxy-7 β -(2-phenoxyacetamido)- Δ^3 -cephem-4-carboxylate (3) [7] and 60 ml (0.96 mol) of MeI in 240 ml of acetone, 22.12 g (160 mmol) of pulverized K_2CO_3 were suspended at 0° and stirred under Ar in an ice/ H_2O bath for 15 h. The K^+ salts were filtered off, the filtrate was evaporated and the residue dissolved in CH_2Cl_2 and washed with 8% aq. NaHCO_3 soln. The crude product as obtained by evaporation of the org. phase was chromatographed on 700 g of silica gel (Merck, 230–400 mesh ASTM). After several, almost empty fractions eluted with toluene, the major 4 α -carboxylate **4** was eluted in many fractions with toluene/AcOEt 19:1 (11.0 g), followed, after several mixed fractions (1.6 g), by the minor 4 β -carboxylate **5** (4.06 g) eluted with toluene/AcOEt 19:1 and 4:1. Crystallization of the latter from $\text{CH}_2\text{Cl}_2/\text{Et}_2\text{O}$ gave 2.86 g of pure **5**; the mother liquors were combined with the above mentioned mixed fractions and rechromatographed on 10 prep. TLC plates ($20 \times 100 \times 0.2$ cm) with toluene/AcOEt 2:1, giving 1.11 g of pure **4** and 0.64 g of pure **5**, increasing the total yield of pure **4** to 12.11 g (57.1%) and that of **5** to 3.50 g (16.5%).

Benzhydryl 4 β -Methyl-3-oxo-7 β -(2-phenoxyacetamido)cepham-4 α -carboxylate⁶⁾ (4). Amorphous foam. R_f (toluene/AcOEt 1:1) 0.53. $[\alpha]_D^{20} = +162.3 \pm 0.9^\circ$ ($c = 1.132$, CHCl_3). IR (CH_2Cl_2): 3400, 1780, 1728, 1696, 1600, 1515, 1496, 1440, 1350, 1230, 1178, 1140, 1080, 1060, 950. $^1\text{H-NMR}$ (400 MHz, CDCl_3): 7.37–6.91 (m , 17 H); 5.37 (dd , $J = 8.0, 4.5$, 1 H); 5.25 (d , $J = 4.5$, 1 H); 4.59 (m , 2 H); 3.41 (d , $J = 15$, 1 H); 3.09 (d , $J = 15$, 1 H); 1.98 (s , 3 H). Anal. calc. for $\text{C}_{29}\text{H}_{26}\text{N}_2\text{O}_6\text{S}$ (530.60): C 65.65, H 4.94, N 5.28, O 18.09, S 6.04; found: C 65.84, H 5.14, N 5.14, O 17.99, S 5.78.

Benzhydryl 4 α -Methyl-3-oxo-7 β -(2-phenoxyacetamido)cepham-4 β -carboxylate⁶⁾ (5). M.p. 151–153° ($\text{CH}_2\text{Cl}_2/\text{Et}_2\text{O}$). R_f (toluene/AcOEt 1:1) 0.46. $[\alpha]_D^{20} = +266.9 \pm 1.0^\circ$ ($c = 0.961$, CHCl_3). IR (CH_2Cl_2): 3414, 1783, 1732, 1698, 1600, 1515, 1496, 1442, 1349, 1239, 1177, 1083, 1062, 957. $^1\text{H-NMR}$ (400 MHz, CDCl_3): 7.37–6.93 (m , 16 H); 6.87 (s , 1 H); 5.33 (dd , $J = 7.5, 4.0$, 1 H); 5.04 (d , $J = 4.0$, 1 H); 4.59 (m , 2 H); 3.70 (d , $J = 14$, 1 H); 2.93 (d , $J = 14$, 1 H); 1.75 (s , 3 H); irradiation at 1.75 (CH_3) $\rightarrow$ NOE at 5.04 (H–C(6)). Anal. calc. for $\text{C}_{29}\text{H}_{26}\text{N}_2\text{O}_6\text{S}$ (530.60): C 65.65, H 4.94, N 5.28, O 18.09, S 6.04; found: C 65.63, H 5.03, N 5.17, O 18.13, S 5.81.

Benzhydryl 3 β -Hydroxy-4 α -methyl-7 β -(2-phenoxyacetamido)cepham-4 β -carboxylate⁶⁾ (6). To a soln. of 4.43 g (8.35 mmol) of **5** in 40 ml of DMF and 10.5 ml of AcOH, 397 mg (10.5 mmol) of NaBH_4 were added in small portions within 20 min while stirring under Ar in an ice/ H_2O bath. After a total of 60 min of stirring at 0–5°, the mixture was evaporated at 40° under the vacuum of an oil pump and the residue in CH_2Cl_2 washed with H_2O and sat. brine; the aq. parts were reextracted with CH_2Cl_2 . The crude product (4.75 g) obtained by evaporation of the combined org. extracts was chromatographed on 200 g of Merck silica gel 60 with toluene/AcOEt 9:1. After several fractions containing mainly the minor **9** (see below), a total of 3.34 g (75.1%) of pure **6** was collected as amorphous foam, enclosing trace amounts of solvents (e.g. toluene), even after drying for several days at 30°/0.1 mbar. R_f (toluene/AcOEt 1:1) 0.39. $[\alpha]_D^{20} = +95.7 \pm 1.0^\circ$ ($c = 1.023$, CHCl_3). IR (CH_2Cl_2): 3550, 3405, 3052, 1779, 1730, 1692, 1599, 1517, 1494, 1440, 1349, 1226, 1156, 1081, 961. $^1\text{H-NMR}$ (400 MHz, CDCl_3): 7.60 (d , $J = 9$, 1 H); 7.40–6.89 (m , 16 H); 5.69 (dd , $J = 9, 4.5$, 1 H); 5.08 (d , $J = 4.5$, 1 H); 4.54 (s , 2 H); 4.06 (dd , $J = 4.5, 1.5$, 1 H); 3.33 (dd , $J = 14.5, 1.5$, 1 H); 2.87 (dd , $J = 14.5, 4.5$, 1 H); 1.46 (s , 3 H); irradiation at 1.46 $\rightarrow$ NOE at 5.08, 4.06, and 3.33. Anal. calc. for $\text{C}_{29}\text{H}_{28}\text{N}_2\text{O}_6\text{S}$ (532.61): C 65.40, H 5.30, N 5.26, S 6.02; found: C 64.88, H 5.50, N 4.99, S 5.83.

In another experiment with 6.15 g of **5**, the early fractions (0.30 g) of the chromatography of the crude product were rechromatographed on Merck silica gel plates in toluene/AcOEt 2:1, yielding 107 mg of benzhydryl 3 α -hydroxy-4 α -methyl-7 β -(2-phenoxyacetamido)cepham-4 β -carboxylate⁶⁾ (**9**) as a minor product. Amorphous foam. R_f (toluene/AcOEt 1:1) 0.44. IR (CH_2Cl_2): 3550, 3400, 1775, 1730, 1690, 1598, 1518, 1492, 1450–1417 ($br.$), 1360–1320 ($br.$), 1221, 1182, 1061, 990. $^1\text{H-NMR}$ (400 MHz, CDCl_3): 7.42–6.90 (m , 17 H); 5.64 (dd , $J = 9, 4$, 1 H); 5.02 (d , $J = 4$, 1 H); 4.52 (s , 2 H); 4.36 (dd , $J = 10, 4$, 1 H); 3.00 (dd , $J = 13, 10$, 1 H); 2.67 (dd , $J = 13, 4$, 1 H); 1.52 (s , 3 H); irradiation at 1.52 $\rightarrow$ NOE at 5.02, 4.36, and 3.00.

⁶⁾ The systematic names of the parent cepham-, Δ^2 -cephem-, and Δ^3 -cephem-4-carboxylic acids are 8-oxo-5-thia-1-azabicyclo[4.2.0]octane-, 8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-, and 8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, respectively.

*Benzhydryl 3β-(Methansulfonyloxy)-4α-methyl-7β-(2-phenoxyacetamido)cepham-4β-carboxylate*⁶) (7). To a soln. of 2.16 g (4.05 mmol) of **6** and of 488 mg (4 mmol) of 4-(dimethylamino)pyridine in 30 ml of pyridine, 3.1 ml (ca. 40 mmol) of MeSCl were slowly added while stirring under Ar at r.t. After a total of 3 h, the mixture (with a crystalline precipitate) was evaporated under high vacuum and the residue in CH₂Cl₂ washed with 8% aq. NaHCO₃ soln. and H₂O; the aq. parts were reextracted with CH₂Cl₂. The residue obtained by evaporation of the combined org. parts (3.2 g) was chromatographed on 100 g of Merck silica gel (230–400 mesh ASTM) with toluene/AcOEt 9:1. After several small fractions containing impurities, a total of 1.49 g (61%) of **7** was collected as a solid foam enclosing solvent residues, even after prolonged drying under high vacuum. *R_f* (toluene/AcOEt 2:1) 0.31. IR (CH₂Cl₂): 3405, 3055, 1791, 1744, 1696, 1600, 1519, 1496, 1454, 1442, 1360, 1240, 1179, 1156, 1084, 1064, 1020, 964, 914. ¹H-NMR (400 MHz, CDCl₃): 7.60 (*d*, *J* = 9, 1 H); 7.48–6.97 (*m*, 15 H); 6.87 (*s*, 1 H); 5.75 (*dd*, *J* = 9, 4, 1 H); 5.10 (*d*, *J* = 4, 1 H); 5.03 (*dd*, *J* = 4, 1, 1 H); 4.54 (*s*, 2 H); 3.41 (*dd*, *J* = 14, 1, 1 H); 3.22 (*dd*, *J* = 14, 4, 1 H); 2.74 (*s*, 3 H); 1.46 (*s*, 3 H). Anal. calc. for C₃₀H₃₀N₂O₈S₂ (610.70): C 59.00, H 4.95, N 4.59, O 20.96, S 10.50; found: C 57.27, H 4.76, N 4.51, O 19.84, S 9.90, Cl 3.30; the analysis fits if 4.0% of residual CH₂Cl₂ is considered: calc. C 57.23, H 4.84, N 4.41, O 20.13, S 10.08, Cl 3.30.

In another mesylation experiment, 608 mg (1.14 mmol) of **6** in 8.5 ml of CH₂Cl₂ was treated at r.t. with 133 μl (ca. 1.71 mmol) of MeSCl in the presence of 277 mg (2.74 mmol) of Et₃N. Workup after 4 h by washing with 8% aq. NaHCO₃ soln. and H₂O and prep. TLC of the crude product (Merck silica gel 60 plates) with toluene/AcOEt 2:1 afforded only 217 mg (31.1%) of **7**; however, 254 mg of another, less mobile compound, *benzhydryl 8-methyl-3-oxo-4-(2-phenoxyacetamido)-2-oxa-6-thia-9-azabicyclo[3.2.2]nonane-8-carboxylate* (**10**), was also isolated. Amorphous foam. *R_f* (toluene/AcOEt 2:1) 0.20. IR (CH₂Cl₂): 3400, 1740, 1690, 1598, 1585, 1508, 1493, 1438, 1382, 1370, 1220, 1120, 1081, 1060, 1000, 912. ¹H-NMR (400 MHz, CDCl₃): 7.42–6.90 (*m*, 17 H); 5.36 (*dd*, *J* = 5, 1, 1 H); 5.10 (*t*, *J* = 2, 1 H); 4.50 (*s*, 2 H); 4.47 (*d*, *J* = 1, 1 H); 3.27 (*m*, 2 H); 1.63 (*s*, 3 H).

The lactone **10** was also detected (by NMR) as a by-product in the NaBH₄ reduction of **5** to **6** (see above).

*Benzhydryl 4α-Methyl-7β-(2-phenoxyacetamido)-Δ²-cephem-4β-carboxylate*⁶) (**8**). A soln. of 6.81 g (21.6 mmol) of Bu₄NF·3 H₂O in 60 ml of THF was dehydrated by standing overnight in a refrigerator (+5°) with molecular sieves (Merck No. 5708; 0.4 nm, pearl form). Of the resulting soln., 30 ml were added to a soln. of 2.20 g (3.6 mmol) of **7** in 72 ml of abs. THF, and the mixture thus obtained was stirred under Ar at r.t. for 3 h. Thereafter, it was diluted with CH₂Cl₂ and washed twice with a buffer soln. (pH 8.0); the aq. parts were reextracted with CH₂Cl₂. The crude product obtained by evaporation of the CH₂Cl₂ (2.2 g) was chromatographed on 100 g of Merck silica gel (230–400 mesh ASTM) using toluene/AcOEt 19:1. First, a mixture (254 mg) of **8** and of its 6,7-*trans* isomer was eluted, followed by 926 mg of unchanged **7**. The mixture of **8** and its isomer was rechromatographed on 12 Merck anal. silica-gel plates in toluene/AcOEt 2:1, yielding 47 mg (4.4%) of the *trans*-isomer and 170 mg (15.8%, based on consumed **7**) of **8**.

8: Solid foam, enclosing solvent residues, even after extended drying at 30°/0.1 mbar. *R_f* (toluene/AcOEt 2:1) 0.38. [α]_D²⁰ = –46.8 ± 1.1° (*c* = 0.923, CHCl₃). IR (CH₂Cl₂): 3409, 3051, 1780, 1744, 1696, 1600, 1518, 1496, 1442, 1372, 1229, 1131, 1084. ¹H-NMR (400 MHz, CDCl₃): 7.35–6.84 (*m*, 17 H); 6.43 (*d*, *J* = 10, 1 H); 5.77 (*d*, *J* = 10, 1 H); 5.61 (*dd*, *J* = 9, 4.5, 1 H); 5.06 (*d*, *J* = 4.5, 1 H); 4.54 (*m*, 2 H); 1.61 (*s*, 3 H). MS (*m/z*): 514 (*M*⁺), 324, 303, 275, 167, 165, 152, 112, 107. Anal. calc. for C₂₉H₂₆N₂O₅S (514.60): C 67.69, H 5.09, N 5.44, S 6.23; found: C 66.33, H 5.10, N 5.25, S 5.94; calc. for 3.17% of enclosed CH₂Cl₂: C 66.33, H 5.00, N 5.26, S 6.03.

6,7-*trans*-Isomer of **8** (absolute configuration at C(6) and C(7) unknown): Solid foam. *R_f* (toluene/AcOEt 2:1) 0.47. IR (CH₂Cl₂): 3400, 1773, 1740, 1691, 1598, 1520, 1495, 1377, 1230, 1130, 1117, 1080. ¹H-NMR (400 MHz, CDCl₃): 7.35–6.92 (*m*, 16 H); 6.81 (*s*, 1 H); 6.48 (*d*, *J* = 10, 1 H); 5.71 (*d*, *J* = 10, 1 H); 4.80 (*dd*, *J* = 7, 2, 1 H); 4.76 (*d*, *J* = 2, 1 H); 4.52 (*s*, 2 H); 1.71 (*s*, 3 H).

1,4α,5,7α,8,9α-Hexahydro-9α-methyl-1,8-dioxo-7β-(2-phenoxyacetamido)-2H,6aaH-azeto[2,1-b][1,2]oxathiazino[5,6-d][1,3]thiazine 3,3-Dioxide (**11**). In the above described preparation of **8**, the polar by-product **11** was isolated in one case by prep. TLC of the crude product. Starting with 183 mg of **7**, 30 mg of **11** were obtained from 5 Merck silica-gel plates (20 × 20 × 0.05 cm) with toluene/AcOEt 2:1. Amorphous foam. *R_f* (toluene/AcOEt 2:1) 0.10. IR (CH₂Cl₂): 3400, 1767, 1740, 1695, 1597, 1510, 1492, 1380, 1358, 1225, 1179, 1160, 1062, 980. ¹H-NMR (400 MHz, CDCl₃): 7.47 (*d*, *J* = 9, 1 H); 7.34–6.99 (*m*, 5 H); 5.64 (*dd*, *J* = 9, 4.5, 1 H); 5.12 (*d*, *J* = 15, 1 H); 5.10 (*d*, *J* = 4.5, 1 H); 4.58 (*m*, 2 H); 4.49 (*dd*, *J* = 4, 1.5, 1 H); 4.36 (*d*, *J* = 15, 1 H); 3.51 (*dd*, *J* = 15.5, 1.5, 1 H); 3.24 (*dd*, *J* = 15.5, 4, 1 H); 1.48 (*s*, 3 H); irradiation at 1.48 → NOE at 5.10, 4.49, and 3.51. ¹³C-NMR (100.6 MHz, CDCl₃): 188.3; 168.9; 163.8; 156.5; 129.7; 122.2; 114.6; 71.0; 66.7; 61.3; 60.3; 57.5; 54.4; 26.7; 16.7.

*Sodium 4α-Methyl-7β-(2-phenoxyacetamido)-Δ²-cephem-4β-carboxylate*⁶) (**1a**). To a soln. of 170 mg (0.33 mmol) of **8** and of 53 mg (0.5 mmol) of anisole in 1.7 ml of CH₂Cl₂, 252 μl (3.3 mmol) of CF₃COOH were added at 0–5°. The resulting mixture was stirred under Ar in an ice/H₂O bath for 2.5 h. Thereafter, it was diluted with toluene and evaporated, and the residue was once more evaporated with CH₂Cl₂/toluene (removal of CF₃COOH).

Trituration of the residue with MeOH and filtration removed a small amount of a white precipitate. The filtrate was evaporated and the residue redissolved in 1.2 ml of MeOH. Then, 0.2 ml of 3M sodium hexanoate in MeOH were added. On addition of Et₂O to the resulting soln., **1a** precipitated. After filtration, it was washed with Et₂O to give 79 mg (64.6%) of a white, microcrystalline material. IR (KBr): 3428 (br.), 3050, 1754, 1680, 1625, 1535, 1496, 1442, 1387, 1355, 1236, 1085. ¹H-NMR (400 MHz, CD₃OD): 7.29–6.96 (*m*, 5 H); 6.28 (*d*, *J* = 10, 1 H); 5.83 (*d*, *J* = 10, 1 H); 5.48 (*d*, *J* = 4, 1 H); 5.04 (*d*, *J* = 4, 1 H); 4.59 (*s*, 2 H); 1.50 (*s*, 3 H); irradiation at 1.50 → NOE at 5.83 and 5.04. FAB-MS (thioglycerol matrix): 371 (*[M + H]⁺*) and several cluster ions, e.g. 393 (*[M + H + Na]⁺*), 479 (*[M + thioglycerol + H]⁺*), 501 (*[479 + Na]⁺*), 763 (*[M + M + Na]⁺*).

Stability tests: *a*) According to ¹H-NMR, a 0.74% soln. of **1a** in a D₂O-phosphate buffer, 'pD' 7.4, remained unchanged at r.t. for 12 days. At 37°, a soln. of **1a** in D₂O was unchanged after 24 h. *b*) *t*_{1/2} at 37°: 50 h in a biological buffer soln., pH 7.4; 2 h in a phosphate buffer soln., pH 3.0; 50 h in human plasma (HPLC evidence).

Benzhydryl 3α-Hydroxy-4β-methyl-7β-(2-phenoxyacetamido)cepham-4α-carboxylate⁶ (14). Reduction of 6.25 g (11.8 mmol) of **4** in a way similar to that described above for the preparation of **6** (60 ml of DMF, 15 ml of AcOH, 840 mg of NaBH₄; 90 min at 0°) gave, after chromatography of the crude product on 300 g of Merck silica gel 60 with toluene/AcOEt 19:1, 3.48 g (55.5%) of **14**. It crystallized from CH₂Cl₂/pentane in white needles including some CH₂Cl₂. M. p. (65° →) 71°. *R*_f (toluene/AcOEt 1:1) 0.52. IR (CH₂Cl₂): 3406, 3053, 1772, 1695, 1599, 1520, 1495, 1441, 1366, 1236, 1173, 1133, 1062, 956. ¹H-NMR (400 MHz, CDCl₃): 7.43–6.92 (*m*, 17 H); 5.55 (*dd*, *J* = 9.5, 4.5, 1 H); 4.86 (*d*, *J* = 4.5, 1 H); 4.54 (*m*, 2 H); 3.83 (*m*, 1 H); 3.45 (*d*, *J* = 10, 1 H); 2.78 (*m*, 2 H); 2.01 (*s*, 3 H). FAB-MS: 533 (*[M + H]⁺*). Anal. calc. for C₂₉H₂₈N₂O₆S (532.61): C 65.40, H 5.30, N 5.26, O 18.02, S 6.02; found: C 64.44, H 5.20, N 5.31, O 17.93, S 6.12, Cl 1.10.

Benzhydryl 3α-(Methansulfonyloxy)-4β-methyl-7β-(2-phenoxyacetamido)cepham-4α-carboxylate⁶ (15). To a soln. of 1.06 g (2.0 mmol) of **14** and 0.67 ml (4.8 mmol) of Et₃N in 15 ml of CH₂Cl₂, 0.23 ml (3 mmol) of mesyl chloride were added at 0° and stirred at r.t. for 75 min. Thereafter, the mixture was diluted with CH₂Cl₂ and successively washed with H₂O and aq. NaHCO₃ soln.; the aq. parts were reextracted with CH₂Cl₂. The crude product as obtained by evaporation of the combined org. parts was chromatographed on 30 g of Merck silica gel 60 with toluene/AcOEt 9:1. A total of 1.04 g (85.2%) of **15** was collected in several fractions as a solid foam retaining traces of residual solvents (e.g. toluene), even after extensive drying under high vacuum. *R*_f (toluene/AcOEt 2:1) 0.43. [*α*_D²⁰] = +77 ± 1° (*c* = 1.035, CHCl₃). IR (CH₂Cl₂): 3406, 3055, 1777, 1734, 1696, 1600, 1519, 1495, 1441, 1351, 1238, 1210, 1178, 1133, 1082, 949, 871, 821. ¹H-NMR (400 MHz, CDCl₃): 7.42–6.93 (*m*, 17 H); 5.55 (*dd*, *J* = 9.5, 4.5, 1 H); 5.32 (*d*, *J* = 4.5, 1 H); 4.84 (*dd*, *J* = 10, 3.5, 1 H); 4.56 (*m*, 2 H); 3.45 (*dd*, *J* = 13.5, 10, 1 H); 2.98 (*dd*, *J* = 13.5, 3.5, 1 H); 2.75 (*s*, 3 H); 2.06 (*s*, 3 H); irradiation at 2.06 → NOE at 4.88; irradiation at 3.45 → NOE at 2.98 and 5.32. FAB-MS: 611 (*[M + H]⁺*). Anal. calc. for C₃₀H₃₀N₂O₈S₂ (610.70): C 59.00, H 4.95, N 4.59, S 10.50; found: C 59.65, H 5.18, N 4.39, S 10.29.

4α-[(Benzhydryloxy)carbonyl]-4β-methyl-7β-(2-phenoxyacetamido)-Δ²-cephem 1-Oxide⁶ (19). To a soln. of 10.0 g (ca. 20 mmol) of benzhydryl 7β-(2-phenoxyacetamido)-Δ²-cephem-4-carboxylate (**17**) [7a] [8] in 200 ml of CH₂Cl₂, 4.22 g of 90% *m*-chloroperbenzoic acid were added in several portions, while stirring in an ice/H₂O bath. After a total of 90 min, the resulting mixture (with precipitated *m*-chlorobenzoic acid) was diluted with more CH₂Cl₂ and successively washed with H₂O, 8% aq. NaHCO₃ soln., and sat. brine; the aq. parts were reextracted with CH₂Cl₂. Evaporation of CH₂Cl₂ from the combined org. parts left a crystalline residue which, after washing with Et₂O, afforded 9.5 g (92%) of white crystals of 4-[(benzhydryloxy)carbonyl]-7β-(2-phenoxyacetamido)-Δ²-cephem-4-carboxylate 1-oxide⁶ (**18**). This material was used in the next step without any further purification. *R*_f (toluene/AcOEt 1:1) 0.19. IR (CH₂Cl₂): 3400, 1791, 1720, 1687, 1635, 1595, 1505, 1485, 1390, 1220, 1160, 1100, 1077, 1055, 1038, 980, 960.

To **18** (13.3 g, 25.7 mmol) in 75 ml of DMF and 625 ml of MeCN, 20.6 ml of MeI were added and stirred with 3.6 g of finely pulverized K₂CO₃ at r.t. After 15 h, more MeI (10 ml) was added and stirring was continued for another 6 h. The resulting brown mixture was evaporated and the residue partitioned between AcOEt and H₂O; the org. phase was washed with sat. brine, and all aq. parts were reextracted with AcOEt. Evaporation of AcOEt from the combined org. parts left a brown foam (15 g) which was chromatographed on 250 g of Merck silic gel 60. After several fractions with toluene/AcOEt 9:1, **19** was eluted with toluene/AcOEt 4:1 and 3:1 as a colourless foam (6.0 g, 43.9%). *R*_f (toluene/AcOEt 1:1) 0.35. IR (CH₂Cl₂): 3360, 1770, 1730, 1680, 1591, 1503, 1482, 1365, 1315, 1204, 1110, 1071, 1052, 1020. ¹H-NMR (360 MHz, CDCl₃): 8.18 (*d*, *J* = 10.2, 1 H); 7.41–6.72 (*m*, 18 H); 6.09 (*dd*, *J* = 10.2, 5, 1 H); 4.58 (*AB*, *J* = 15, 2 H); 4.52 (*d*, *J* = 5, 1 H); 2.13 (*s*, 3 H).

Benzhydryl 4β-Methyl-7β-(2-phenoxyacetamido)-Δ²-cephem-4α-carboxylate⁶ (16). To a soln. of 5.5 g (10.37 mmol) of **19** in 45 ml of DMF, 1.82 ml (20.86 mmol) of PCl₃ were slowly added at –10° and stirred under Ar at –10° for another 15 min. Then, the mixture was poured on crashed ice, the product extracted into AcOEt, and the org. part successively washed with 8% aq. NaHCO₃ soln., H₂O, and sat. brine; all aq. parts were reextracted with

AcOEt. Evaporation of the combined org. extracts, finally under high vacuum, afforded 5.2 g of a brown residue which was chromatographed on 150 g of *Merck* silica gel 60 using toluene/AcOEt 9:1. The title ester was collected in several fractions as a solid foam (4.2 g, 78.7%), crystallizing from AcOEt on addition of petroleum ether. M.p. 98–102° (AcOEt/petroleum ether). R_f (toluene/AcOEt 1:1) 0.59. IR (CH₂Cl₂): 3400, 1775, 1740, 1694, 1599, 1518, 1494, 1440, 1382, 1245, 1228, 1124, 1082, 1065. ¹H-NMR (100 MHz, CDCl₃): 7.46–6.80 (*m*, 17 H); 6.3 (*d*, *J* = 10.1, 1 H); 5.82 (*d*, *J* = 10.1, 1 H); 5.7 (*dd*, *J* = 9, 5, 1 H); 5.14 (*d*, *J* = 5, 1 H); 4.56 (*s*, 2 H); 1.94 (*s*, 3 H). Anal. calc. for C₂₉H₂₆N₂O₅S (514.60): C 67.69, H 5.09, N 5.44, S 6.23; found: C 67.84, H 5.25, N 5.59, S 6.22.

Crystal-Structure Analysis of 16. Crystal Data: Monoclinic, space group *C*₂, *a* = 17.997(6), *b* = 10.074(5), *c* = 15.918(6) Å, β = 91.22(5)°, *Z* = 4. A *Philips PW1100* automatic diffractometer was used for data collection with MoK α radiation and graphite monochromator. The intensities of 4462 independent reflections with $\theta < 30^\circ$ were measured of which 3653 were classified as observed with $I > 2\sigma(I)$. The structure was solved by direct methods using the MULTAN 78 program [10]. From a difference *Fourier* map, 21 of 26 H-atoms were found, the coordinates of the remaining were calculated assuming tetrahedral geometry. The structure was refined by full matrix least squares calculations with anisotropic (isotropic for H-atoms) thermal parameters to a final *R* value of 0.081.

Table 2. Positional Parameters and their Estimated Standard Deviations for Non-H-Atoms of 16

Atom	<i>x</i>	<i>y</i>	<i>z</i>	Atom	<i>x</i>	<i>y</i>	<i>z</i>
S(1)	0.3016(1)	0.3790(3)	0.0432(1)	C(20)	0.2370(4)	0.6867(7)	–0.3415(4)
C(2)	0.3177(5)	0.5521(9)	0.0365(6)	C(21)	0.2901(6)	0.735(1)	–0.3980(6)
C(3)	0.3427(4)	0.6203(8)	–0.0291(5)	C(22)	0.2882(7)	0.679(1)	–0.4792(7)
C(4)	0.3627(4)	0.5623(7)	–0.1123(4)	C(23)	0.2359(7)	0.583(1)	–0.5076(7)
N(5)	0.3738(3)	0.4183(5)	–0.1040(3)	C(24)	0.1854(6)	0.540(1)	–0.4506(6)
C(6)	0.3233(4)	0.3253(8)	–0.0623(5)	C(25)	0.1863(6)	0.593(1)	–0.3712(6)
C(7)	0.3876(4)	0.2204(7)	–0.0650(5)	N(26)	0.4087(3)	0.1537(6)	0.0101(4)
C(8)	0.4366(4)	0.3361(7)	–0.0929(4)	C(27)	0.4053(8)	0.0204(5)	0.0116(4)
C(9)	0.4350(5)	0.6271(8)	–0.1424(6)	O(28)	0.3914(3)	–0.0510(6)	–0.0484(5)
C(10)	0.2957(4)	0.5806(7)	–0.1740(4)	C(29)	0.4251(4)	–0.0434(7)	0.0954(4)
O(11)	0.2486(3)	0.4997(6)	–0.1863(4)	O(30)	0.4509(3)	0.0542(5)	0.1524(3)
O(12)	0.2965(2)	0.7033(5)	–0.2058(3)	C(31)	0.4768(4)	0.0126(8)	0.2287(5)
C(13)	0.2301(4)	0.7424(7)	–0.2533(4)	C(32)	0.5051(5)	0.106(1)	0.2812(5)
C(14)	0.2262(4)	0.8915(7)	–0.2556(4)	C(33)	0.5320(5)	0.074(1)	0.3617(6)
C(15)	0.2864(4)	0.9732(8)	–0.2472(4)	C(34)	0.5282(5)	–0.055(1)	0.3897(6)
C(16)	0.2760(5)	1.1109(8)	–0.2539(5)	C(35)	0.4992(5)	–0.149(1)	0.3373(5)
C(17)	0.2069(5)	1.1710(9)	–0.2660(6)	C(36)	0.4718(4)	–0.1144(9)	0.2570(5)
C(18)	0.1499(6)	1.081(1)	–0.2734(7)	O(37)	0.5016(3)	0.3536(6)	–0.1068(3)
C(19)	0.1580(5)	0.9464(9)	–0.2670(6)				

Table 3. Positional Parameters and their Estimated Standard Deviations for H-Atoms of 16

Atom	<i>x</i>	<i>y</i>	<i>z</i>	Atom	<i>x</i>	<i>y</i>	<i>z</i>
H(38)	0.305(4)	0.614(9)	0.089(5)	H(51)	0.328(5)	0.808(9)	–0.374(5)
H(39)	0.349(5)	0.724(9)	–0.020(5)	H(52)	0.327(5)	0.713(9)	–0.525(5)
H(40)	0.382(4)	0.134(9)	–0.106(5)	H(53)	0.234(5)	0.539(9)	–0.565(5)
H(41)	0.271(5)	0.306(9)	–0.095(5)	H(54)	0.145(5)	0.465(9)	–0.472(5)
H(42)	0.450(4)	0.587(9)	–0.202(5)	H(55)	0.148(5)	0.563(9)	–0.325(5)
H(43)	0.479(5)	0.609(9)	–0.097(5)	H(56)	0.421(5)	0.206(9)	0.066(5)
H(44)	0.426(5)	0.734(9)	–0.148(5)	H(57)	0.468(5)	–0.116(9)	0.087(5)
H(45)	0.182(5)	0.701(9)	–0.223(5)	H(58)	0.376(5)	–0.091(9)	0.121(5)
H(46)	0.339(5)	0.930(9)	–0.239(5)	H(59)	0.509(5)	0.205(9)	0.259(5)
H(47)	0.324(5)	1.175(9)	–0.247(5)	H(60)	0.555(5)	0.148(9)	0.402(5)
H(48)	0.199(5)	1.265(9)	–0.271(5)	H(61)	0.547(5)	–0.083(9)	0.448(5)
H(49)	0.095(5)	1.119(9)	–0.285(5)	H(62)	0.496(5)	–0.250(9)	0.358(5)
H(50)	0.110(5)	0.883(9)	–0.274(5)	H(63)	0.450(5)	–0.189(9)	0.216(5)

Table 4. Bond Distances [$\text{\AA}$] in **16**^{a)}

S(1)–C(2)	1.771(7)	C(10)–O(12)	1.335(9)	C(23)–C(24)	1.37(2)
S(1)–C(6)	1.817(7)	O(12)–C(13)	1.453(8)	C(24)–C(25)	1.37(2)
C(2)–C(3)	1.34(1)	C(13)–C(14)	1.50(1)	N(26)–C(27)	1.344(9)
C(3)–C(4)	1.45(1)	C(13)–C(20)	1.52(1)	C(27)–O(28)	1.219(9)
C(4)–N(5)	1.47(1)	C(14)–C(15)	1.36(1)	C(27)–C(29)	1.52(1)
C(4)–C(9)	1.54(1)	C(14)–C(19)	1.35(1)	C(29)–O(30)	1.410(9)
C(4)–C(10)	1.55(1)	C(15)–C(16)	1.40(1)	O(30)–C(31)	1.356(9)
N(5)–C(6)	1.471(9)	C(16)–C(17)	1.39(1)	C(31)–C(32)	1.35(1)
N(5)–C(8)	1.409(9)	C(17)–C(18)	1.37(1)	C(31)–C(36)	1.36(1)
C(6)–C(7)	1.57(1)	C(18)–C(19)	1.36(2)	C(32)–C(33)	1.40(1)
C(7)–C(8)	1.53(1)	C(20)–C(21)	1.41(1)	C(33)–C(34)	1.38(2)
C(7)–N(26)	1.417(9)	C(20)–C(25)	1.39(1)	C(34)–C(35)	1.36(2)
C(8)–O(37)	1.208(9)	C(21)–C(22)	1.41(2)	C(35)–C(36)	1.40(1)
C(10)–O(11)	1.189(9)	C(22)–C(23)	1.42(2)		

^{a)} Numbers in parentheses are estimated standard deviations in the least significant digits.

*Sodium 4β-Methyl-7β-(2-phenoxyacetamido)-Δ²-cephem-4α-carboxylate*⁶⁾ (**2a**). To a soln. of 1.03 g (2 mmol) of **16** and of 318 mg (2.94 mmol) of anisole in 10 ml of CH_2Cl_2 , 1.5 ml of CF_3COOH were added, while cooling the mixture in an ice/ H_2O bath. After 2.5 h at 0–5° and 0.5 h at r.t., toluene was added and the mixture evaporated, finally once more with toluene/ CHCl_3 1:1 and with Et_2O . The residue thus obtained (1.3 g) was dissolved in a small volume of MeOH, and 1.3 ml of 3M sodium hexanoate in MeOH was added. Addition of Et_2O at 0° caused precipitation of **2a** as a white, microcrystalline powder which was washed with Et_2O (540 mg, 73%). IR (nujol): 3400 (br.), 1750 (br.), 1680 (br.), 1600, 1530, 1495, 1390, 1354, 1240, 1211, 1065. ¹H-NMR (360 MHz, D_2O): 7.43–7.00 (m, 5 H); 6.27 (d, $J = 10.2$, 1 H); 5.95 (d, $J = 10.2$, 1 H); 5.38 (d, $J = 5$, 1 H); 5.12 (d, $J = 5$, 1 H); 4.76 (s, 2 H); 1.76 (s, 3 H).

Stability tests: a) According to ¹H-NMR, a 0.74% soln. of **2a** in a D_2O -phosphate buffer, 'pD' 7.4, remained unchanged at r.t. over 12 days. b) $t_{1/2}$ at 37°: 50 h in a biological buffer soln., pH 7.4; 50 h in a phosphate buffer, pH 3.0; 50 h in human plasma (all HPLC evidence).

Benzhydryl 3-Methoxy-7β-(2-phenoxyacetamido)-Δ²-cephem-4α- (**20**) *and -4β-carboxylate*⁶⁾ (**21**). A soln. of 13.26 g (25 mmol) of benzhydryl 3-methoxy-7β-(2-phenoxyacetamido)-Δ³-cephem-4-carboxylate (**24**) [7a] in 100 ml of CH_2Cl_2 containing 101 mg (1.0 mmol) of Et_3N was stirred at r.t. for 6 h. The resulting equilibration mixture was washed 3 times with 100 ml each of 0.01N aq. HCl and the org. part evaporated to a foam (13 g). A part (8.0 g) of this residue was chromatographed on 220 g of Merck silica gel 60 with toluene/AcOEt/AcOH 90:10:0.5. Successively, **20** (5.69 g, 71%) and **21** (0.16 g, 2.0%) were eluted followed by the unchanged **24** (1.75 g, 22%). No other products were detected under these mild isomerization conditions.

For the preparation of a larger amount of **21**, 350 g of **24** were isomerized as above. From the crude product, most of **20** and **24** were crystallized by addition of 3 l of Et_2O to the oily material. The crystals were filtered off, the mother liquor – enriched in **21** – was evaporated and the residue (16 g) chromatographed as described above, yielding 6.37 g (1.8%) of pure **21**.

*Benzhydryl 3-Methoxy-7β-(2-phenoxyacetamido)-Δ²-cephem-4α-carboxylate*⁶⁾ (**20**). M.p. 121–122° (EtOH/toluene 4:1). R_f (toluene/AcOEt 4:1) 0.44. $[\alpha]_D^{20} = +281 \pm 1.0^\circ$ ($c = 1.042$, dioxane). IR (CH_2Cl_2): 3404, 3056, 1785, 1747, 1696, 1600, 1519, 1495, 1440, 1324, 1209, 1163, 1083, 1064, 988, 838. ¹H-NMR (100 MHz, CDCl_3): 7.58–6.86 (m, 17 H); 5.70 (dd, $J = 9$, 5, 1 H); 5.26 (d, $J = 5$, 1 H); 5.12 (d, $J = 1$, 1 H); 5.0 (d, $J = 1$, 1 H); 4.55 (s, 2 H); 3.52 (s, 3 H). ¹³C-NMR (25.2 MHz, CDCl_3 ; only relevant resonances): 87.7 (C(2), 1 C); 143.3 (C(3), 1 C); 51.8 (C(4), 1 C); 53.8 (C(6), 1 C); 58.9 (C(7), 1 C); 55 (CH_3O , 1 C). Anal. calc. for $\text{C}_{29}\text{H}_{26}\text{N}_2\text{O}_6\text{S}$ (530.60): C 65.65, H 4.94, N 5.28, S 6.04; found: C 65.76, H 4.90, N 5.43, S 5.93.

*Benzhydryl 3-Methoxy-7β-(2-phenoxyacetamido)-Δ²-cephem-4β-carboxylate*⁶⁾ (**21**). Amorphous foam. R_f (toluene/AcOEt 4:1) 0.27. $[\alpha]_D^{20} = -3.6 \pm 0.9^\circ$ ($c = 1.133$, dioxane). IR (CH_2Cl_2): 3407, 3051, 1785, 1750, 1695, 1600, 1518, 1495, 1440, 1209, 1177, 1116, 1083, 1064, 1005, 837. ¹H-NMR (360 MHz, CDCl_3): 7.38–6.86 (m, 18 H); 5.63 (ddd, $J = 10$, 5, 2, 1 H); 5.32 (s, 1 H); 5.06 (d, $J = 5$, 1 H); 4.61 (d, $J = 2$, 1 H); 4.54 (s, 2 H); 3.50 (s, 3 H). ¹³C-NMR (90.5 MHz, CDCl_3 ; only relevant resonances): 88.9 (C(2), 1 C); 147.9 (C(3), 1 C); 56.4 (C(4), 1 C); 57.3 (C(6), 1 C); 57.8 (C(7), 1 C); 55.3 (CHO_3 , 1 C). FAB-MS: 531 ($[M + \text{H}]^+$). Anal. calc. for $\text{C}_{29}\text{H}_{26}\text{N}_2\text{O}_6\text{S}$ (530.60): C 65.65, H 4.94, N 5.28, S 6.04; found: C 65.76, H 5.07, N 5.12, S 5.80.

*3-Methoxy-7 β -(2-phenoxyacetamido)- Δ^2 -cephem-4 α -carboxylic Acid*⁶⁾ (**22**). To a stirred soln. of 13.26 g (25.0 mmol) of **20** and 6.0 g (55.6 mmol) of anisole in 25 ml of CH₂Cl₂, 44.5 g (390 mmol) of CF₃COOH was added at 0° within 10 min. After another 45 min of stirring at 0°, the product was precipitated by adding 200 ml of (i-Pr)₂O and cooling. The crude **22** was filtered off, washed with (i-Pr)₂O, and dried *i.v.* at r.t. (8.95 g). The product was suspended in 80 ml of CH₂Cl₂, filtered, and crystallized from i-PrOH affording 5.96 g (65%) of pure **22** as white crystals. M.p. 178–179° (dec.). *R*_f (CH₂Cl₂/MeOH/AcOH 75:5:0.5) 0.33. [α]_D²⁰ = 327.3 $\pm$ 0.9° (*c* = 1.081, dioxane). IR (KBr): 3305, 1768, 1725, 1689, 1623, 1597, 1535, 1488, 1443, 1330, 1230, 1205, 1165, 1063, 1015, 763, 698. ¹H-NMR (360 MHz, (D₆)DMSO): 13.5 (br. *s*, 1 H); 9.08 (*d*, *J* = 10, 1 H); 7.35–6.92 (*m*, 15 H); 5.55 (*s*, 1 H); 5.50 (*dd*, *J* = 10, 5, 1 H); 5.17 (*d*, *J* = 5, 1 H); 4.70 (*s*, 1 H); 4.65 (*AB*, *J* = 15, 2 H); 3.55 (*s*, 3 H). ¹³C-NMR (90.5 MHz, (D₆)DMSO): 88.2 (C(2), 1 C); 143.5 (C(3), 1 C); 51.8 (C(4), 1 C); 53.4 (C(6), 1 C); 59.5 (C(7), 1 C); 164.5 (C(8), 1 C); the signals due to the side chains are not listed. FAB-MS: 365 ([*M* + H]⁺). Anal. calc. for C₁₆H₁₆N₂O₆S (364.37): C 52.74, H 4.43, N 7.69, S 8.80; found: C 52.61, H 4.45, N 7.72, S 8.83.

Stability tests: *t*_{1/2} at 37°: 100 h in a biological buffer, pH 7.4; 9 h in human plasma (both HPLC evidence).

*3-Methoxy-7 β -(2-phenoxyacetamido)- Δ^2 -cephem-4 β -carboxylic Acid*⁶⁾ (**23**). As for **22** with 4.61 g (8.7 mmol) of **21**, 2.04 g (18.9 mmol) of anisole in 9 ml of CH₂Cl₂, and 15.4 g (135 mmol) of CF₃COOH. The crude **23** was precipitated by slow addition of 215 ml of (i-Pr)₂O while keeping the temp. below 5°. The white-yellow crystals (2.50 g) obtained by filtration were recrystallized from CH₂Cl₂ yielding 2.15 g (68%) of **23** as small, white crystals. M.p. 161–162° (dec.). *R*_f (CH₂Cl₂/MeOH/AcOH 75:25:0.5) 0.33. [α]_D²⁰ = +50.3 $\pm$ 0.9° (*c* = 1.077, dioxane). IR (KBr): 3315, 1777, 1752, 1675, 1617, 1600, 1542, 1495, 1400, 1210, 1175, 1004, 756, 690. ¹H-NMR (360 MHz, CDCl₃/CD₃OD): 7.38–6.97 (*m*, 5 H); 5.63 (*dd*, *J* = 5, 2, 1 H); 5.45 (*s*, 1 H); 5.10 (*d*, *J* = 5, 1 H); 4.55 (*d*, *J* = 2, 1 H); 3.63 (*s*, 3 H); 4.60 (*s*, masked by the HOD signal, 2 H). ¹³C-NMR (90.5 MHz, (D₆)DMSO): 87.7 (C(2), 1 C); 145.9 (C(3), 1 C); 55.8 (C(4), 1 C); 55.5 (C(6), 1 C); 57.8 (C(7), 1 C); 166.2 (C(8), 1 C); the remaining signals are not listed. FAB-MS: 365 ([*M* + H]⁺). Anal. calc. for C₁₆H₁₆N₂O₆S (364.37): C 52.74, H 4.43, N 7.69, S 8.80; found: C 52.03, H 4.48, N 7.72, S 8.40.

Stability tests: *t*_{1/2} at 37°: > 100 h in a biological buffer, pH 7.4; 50 h in human plasma (both HPLC evidence).

REFERENCES

- [1] D. J. Tipper, J. L. Strominger, *Proc. Natl. Acad. Sci. U.S.A.* **1965**, *54*, 1133.
- [2] R. R. Yocum, D. J. Waxman, J. R. Rasmussen, J. L. Strominger, *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 2730.
- [3] J. M. Ghuysen, J. M. Frère, M. Leyh-Bouille, J. Coyette, J. Dusart, M. Nguyen-Disteche, *Annu. Rev. Biochem.* **1979**, *48*, 73.
- [4] J. M. Frère, J. A. Kelly, D. Klein, J. M. Ghuysen, *Biochem. J.* **1982**, *203*, 223.
- [5] N. C. Cohen, *J. Med. Chem.* **1983**, *28*, 259.
- [6] N. C. Cohen, P. Colin, G. Lemoine, *Tetrahedron* **1981**, *37*, 1711.
- [7] a) R. Scartazzini, H. Bickel, *Helv. Chim. Acta* **1974**, *57*, 1919; b) Publ. DT OLS 2331 133, 2331 148, *Ciba-Geigy Ltd. (CA: 1974, 80, 409, 83018, 83019)*.
- [8] G. Sedelmeier, R. Scartazzini, to *Ciba-Geigy Ltd.*, U.S. patent specification 4436 903, 13.3.1984.
- [9] P. Wirz, H. Fuhrer, unpublished results.
- [10] P. Main, S. E. Hull, L. Lessinger, G. Germain, J. P. Declercq, M. M. Woolfson (Dept. of Physics, University of York, 1978), A system of computer programmes for the automatic solution of crystal structures from X-ray diffraction data.